Online Resource for:

Novel Individualized Power Training Protocol Preserves Physical Function in Adult and Older Mice

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Methodology

Training Details (**Figure 1**).

The following is a more detailed description of how the power training protocol was designed and performed. See Table S1 for definitions of human exercise principles that were incorporated into this new model.

Acclimation training

In the first training session of the acclimation training (**Figure 1C**) the mice were introduced to the powered running wheel (**Figure 1B**). The powered running wheel was set at a low speed (4 rpm) and the mice quickly learned to walk on the wheel. Next, during the following training session, the mice were trained to accept the weight harness (**Figure 1A**). In order to have a mouse accept the weight harness successfully, a modified single banded harness with no weights was placed over their shoulders and then removed after a short period (~5 seconds). This step was repeated 3 times.

In the following training session, the harness (without weights) was placed on the mouse and the mouse was put into the powered running wheel (4 rpm) for 30 seconds. Once again, this step was repeated 3 times. On the last day of acclimation training the mice wore the harness with a light weight (3.2g) and were then placed in the running wheel for 3 repetitions of 30 seconds at 4 rpm. This last day of acclimation training was also the first session of the individualized power training period described below.

Individualized Power Training

The mice initially trained for the first week with the very light weight (3.2g) and a single band harness. After the third day of using 3.2g, the mice were introduced to the double-banded harness (**Figure 1A**) that was used throughout the remainder of the training. During this session, the weight in the harness was increased to 4.5 g. At this stage of the training, the weights placed in the harness increased 10-15% every 3rd to 5th training session (depending upon the progression of the mice cohort) with the goal of carrying ~50% body mass in the harness at the end of the training period.

Importantly, the intensity of the exercise was *individualized* such that the mice ran on the running wheel at variable speeds according to their progression (4-10 rpm). The optimal goal for each set was to have the mouse "fail" (failure is defined below) while running for less than 60 seconds (~30 to no more than 75 seconds) by increasing the rpm to make running with the weight more difficult. We choose 60 seconds as our target goal for training time because the mice would be using primarily anaerobic pathways to fuel the exercise bout, followed by aerobic during recovery. This would closely mimic similar human styles of training. Because the training was individualized the time, distance, and weight were recorded for each set during every training session.

Failure

Failure was defined in 3 ways: *1)* the mouse was incapable of maintaining the rpm in the running wheel. Specifically, the inability to maintain the velocity resulted in the vertical position of the mouse at the back of the wheel. *2)* The mouse stumbled 3 times. We

define “stumble” as losing balance to a degree that gait is disrupted. *3)* The mouse refused to run and grasped the grid of the running wheel or threw off the harness (note, the mice participate voluntarily and can refuse to participate). The first time the vertical position of the mouse was observed, the mouse grasped the grid or the mouse stumbled (first and second time), the rpm of the running wheel was adjusted to a lower speed. If the vertical position was observed a second time or the mouse stumbled a 3rd time, the training set was considered complete (went to failure).

Noncompliance

A mouse was considered noncompliant when he refused to run or removed his harness. A mouse was given 3 opportunities to participate successfully in the specific training session. If not successful, the mouse was excused. Because of this voluntary aspect of the training, if a mouse needed extra time to rest it could refuse to train for a session without consequence to the study. The protocol is designed such that if a mouse were to refuse to perform for 3 consecutive sessions, it would be removed from the study. No mice had to be removed from the current studies for repetitive noncompliance.

Results

No Change in Relative Body Mass Between Groups

There was no change with the intervention in the relative proportion of mass between the control and exercise groups (**Table S3**). Specifically, at baseline, body mass was significantly different between the two adult groups with the control mice (32.2±0.8 g) being 8% heavier, p=0.037, than the trained mice (29.9±0.4 g). The mean mass of the adult control after the intervention (40.4±1.9 g) was 9% greater than the mass of the adult trained group (37.1±1.2 g), p=0.001. There was also no change in the older control, with a nonsignificant 3% mass lost in the older exercise. Note however, the older trained mice lost on average, 19% body fat (see **Fig 6**), thus quite a bit of lean mass was added.

Discussion

Discussion Part 1 Other Animal Models of Exercise and Muscle Hypertrophy

To our knowledge, no representative mouse models of individualized non-invasive, voluntary, and physiological progressive power training exist for the mouse. In order to evaluate the efficacy and safety of novel interventions and their synergistic effects with power training prior to clinical testing in humans, an animal model of voluntary power training is needed. As we are defining power training as a combination of aerobic and resistance training with an emphasis on moving weights at high velocity, it is important to acknowledge current models of exercise and hypertrophy already established. The literature has numerous examples of rodent exercise, and following is a brief review of some pertinent models.

In the current study we define voluntary participation as performance of the activity without external coercion (e.g. shock), with the opportunity to refuse to perform the task. This voluntary aspect is very important to our protocol, because we do not coerce the mice to perform with a shock or use starvation to motivate them to seek food as some other protocols do. Other voluntary exercise models do exist. For example, to mimic endurance exercise training, some research teams adopt voluntary wheel running (VWR) (Graber 2015). This is truly voluntary, as the mice chose whether or not to exercise, for how long and at what intensity. They have a wheel in their cages and can use it or not according to their whim. However, this means that the mice might not exert themselves to the highest level. VWR is a well-documented aerobic exercise model, but it has been adapted by some researchers into an approximation of resistance/power training. In this model, the mouse must overcome either increasing friction to turn the wheel or increasing momentum to get the wheel started (Call 2010). However, in this method, while very useful, the mice do not follow a specific or designated training program, as would humans training in the gym or in human exercise studies. The mice simply hop onto the wheel whenever, for how long, and for the intensity that they chose so this is less representative of human exercise trials that have specific training programs.

There are few, if any, resistance training models in the mouse, but quite a few models of resistance training do exist in the rat such as: a squat rack mimic device, first characterized by Tamaki and colleagues (Tamaki 1992, Krisan 2004, Drummond 2010), a weighted backpack and standing model (Farrell 1999, Fluckey 1995) or the tail-weight ladder climb (Deschenes 2000). However, these models are not fully voluntary as participation is induced via operant condition (punishment avoidance) in the squat and

backpack models with an electric shock to either the tail or the feet, respectively, or in the ladder climb with a spray of cold water. Other research has used food reward as an alternative to punishment for conditioning, but in these models the rats must be food restricted in order to induce hunger (Wirth 2003), which involves its own external stressors.

It is also important to acknowledge there are excellent models of muscle hypertrophy (Always 2005, Cholewa 2014), such as synergistic ablation (Timson 1985) or electrical stimulation (Ryan 2010), that some groups use to approximate resistance exercise stimulus. Available mouse models of hypertrophy, however, are not optimal models of voluntary resistance training because an optimal translational model follows principles of biomechanics, nerve and muscle physiology (e.g. fiber recruitment patterns), and mimics voluntary progressive training in humans. Moreover, these models are associated with increased stress, multiple bouts of anesthesia, result in abnormal muscle hypertrophy, and can cause muscle injury (Always 2005, Cholewa 2014, Timson 1985, Ryan 2010). Therefore, the need exists for a mouse model that closely approximates human power training exercise, is voluntary, and follows physiological principles (e.g. the size principle of neuromuscular activation).

Since, current hypertrophy models used to investigate cellular mechanisms in mice are less representative of voluntary human training, and there seem to be as yet no well-established power training model, we intended to produce and validate a voluntary mouse exercise protocol that mimics human power training as would be performed in the gymnasium. Such training improves performance and physiological parameters including:

muscle strength, endurance, power output, speed, balance, coordination, motor performance, and induces hypertrophy (Kraemer 2000, Kramer 2005). Thus, we hypothesized that many of these same adaptations would occur in our trained mice, and, if so, would successfully validate the protocol as a mimic of human exercise.

Discussion Part 2 Training Specificity

Training specificity refers to the actions of an exercise producing adaptations that facilitate functional improvement in activities similar to the exercise (Morrissey 1995). For example, in order to become better adapted for sprinting, practicing by running at a rapid pace induces more positive effects than would long-distance swimming. Specificity can also refer to the muscle groups targeted by an exercise. For example, biceps curls activate, stimulate and induce plasticity in the biceps (arm flexor), but would do little or nothing to the gastrocnemius (plantar flexor). Hence, in the current study training specificity refers to the mode of exercise (running with a weighted pack), which muscles are influenced, and what functional changes would be expected to occur. Specifically, the outcome measures more closely related to the exercise modality were expected to have greater relative change after training (rotarod would improve more than the inverted cling grip test). The muscle groups used for plantar flexion (gastrocnemius complex consisting of the gastrocnemius, soleus and plantaris) or leg extension at the knee (quadriceps) would be the most affected muscles. Thus, within the limits of training specificity we expected to see evidence of improvement in the outcome measures we selected as an indication that our training protocol for mice was indeed a mimic of human weight training.

Supplemental Tables

Term	Explanation/Description	Application in Study
Progressive Resistance	Exercise performed with increasingly heavier weights over time	Increased weights every 3-5 sessions
Progressive Intensity	Exercise performed at a faster pace or with reduced resting time	~4-10 rpm, by individual ability
Rest Period	Recovery time between sets, a typical rest period for strength enhancement is 4-5 minutes	4-5 minutes rest
Frequency	Number of exercise sessions performed each week	1 session per day, 3 days per week
Sets	1 continuous bout of numerous repetitions of exercise	4-5 sets per session
Set Duration	Number of repetitions per set, Humans: hypertrophy 8-15 repetitions, power 3-6 repetitions, usually not more than 1 minute (15 reps at ~4 seconds)	~30 to <60 seconds, anaerobic, to equal typical human set duration
Training Period	Length of training. Many human studies are 3-4 months. First 1-4 weeks, mainly neural adaptions. Hypertrophy main effect after 4 weeks.	12 weeks
Warm-up Sets	Initial sets performed at very light weight and/or intensity to prevent injury and prime for heavier lifting	1 or 2 warm-up sets per session
Failure	The last repetition of an exercise that can be safely performed in good form	Stumble 3x or reach vertical position twice

Table S1 Training Principles used in Power Training Protocol

Training Parameter	Definition	Equation
Training Force (mN)	Total mass of the mouse plus weight used in each session.	*Mass in grams (mouse mass + weight and harness) * acceleration (g)*
Normalized Training Force (mN/gbm)	Total weight the mice lifted (including body mass) per gram of body mass.	*Training Force/ grams of body mass (gbm)*
Training Power (mW)	Work performed per second.	*Training Power (mW) = Work (mJ) / time (s) [Work (mJ) = Training force (mN) * distance run (m)];*
Normalized Training Power (mW/gbm)	Power produced per gram of body mass.	*Training Power / gbm*

Table S2 Training Physiology Definitions Units: mN = milliNewtons, g = acceleration due to gravity 9.8 meters/second, gbm = grams of body mass, mW = milliWatts, mJ = milliJoules

	Unit	Adult Control	Adult Exercise	Old Control	Old Exercise	ME Age (p)	ME Trained (p)	ME Inter. (p)
Body Mass initial	g	32.6±0.7 a	29.9±0.9 b	32.7±0.7 a	34.0±0.8 a	**0.014**	0.368	**0.018**
Body Mass at sac.	g	40.9±0.9 a	37.1±0.4 a	32.3±0.6 b	32.9±0.9 b	**0.001**	0.359	0.197
Body Mass change	%	24.7±4.0 a	24.4±11.6 a	-1.4±2.5 b	-3.3±2.8 b	**<0.001**	0.765	0.816
BMI at sac.	kg/m^2	4.0±0.1 a	3.7±0.1 a	3.3±0.1 b	3.5±0.1 b	**0.001**	0.853	**0.041**
SOL Mass*	mg	12.6±0.7 a	14.5±0.8 b	9.5±0.6 c	10.9±0.5 c‡	**<0.001**	**0.016**	0.134
SOL Mass/gbm	mg/ gbm	0.31±0.01 a	0.39±0.01 b	0.30±0.02 a§	0.32±0.03 a	0.160	**0.009**	0.465
SOL Fiber Length	mm	8.2±0.2 a	8.7±0.2 a	7.8±0.2 a§	8.4±0.1 a‡	**0.093**	**0.020**	0.771
SOL PCSA	mm^2	1.00±0.03 a	1.10±0.06 a	0.80±0.04 b	0.96±0.04 b	**<0.001**	**0.088**	0.661
P_0	mN	246.9±12.9 a	249.5±9.9 a	178.2±9. c§	184.6±7.3 c§	**<0.001**	0.440	0.583
P_0/gbm	mN/ gbm	7.6±0.3 a	8.4±0.3 b	5.4±0.3 c§	5.8±0.3 c§	**<0.001**	**0.071**	0.473

Table S3 Animal Characteristics ME Age = main effect of age, ME Trained = main effect of training, ME Inter. = interaction effect of age*training (numbers in columns = p-value from 2x2 ANOVA with bold indicating significance); different letters indicate differences at p<0.10; "‡" ≠ Older Control, p<0.05; "§" ≠ Adult Exercise, p<0.05; "¥" = Adult Exercise; BMI = body mass index; sac.= sacrifice; gbm = grams of body mass; PCSA = physiological cross sectional area; P_0 = maximum tetanic force; g = grams; mg = milligram; mN = milliNewton; kg/M^2 = kilogram divided by meters squared.

Velocity fl/s	AC	AE	OC	OE	2x2 ANOVA p-value	Age	Training	Inter.
V_{max}	4.5±0.3 a	4.2±0.2 a	3.7±0.2 b¥	3.7±0.2 b¥	**<0.001**	**0.014**	0.549	0.488
10%P_0	2.44±0.096 a	2.59±0.139 a	2.14±0.112 b	2.17±0.116 a	**<0.001**	**0.005**	0.447	0.618
20%P_0	1.61±0.061 a	1.74±0.094 a	1.43±0.078 b	1.45±0.071 a§	**<0.001**	**0.006**	0.360	0.515
30%P_0	1.13±0.041 a	1.24±0.068 a	0.99±0.052 b	1.02±0.051 a§	**<0.001**	**0.002**	0.214	0.479
40%P_0	0.81±0.029 a	0.90±0.050 a	0.71±0.038 b	0.73±0.036 a§	**<0.001**	**0.001**	0.155	0.363
50%P_0	0.57±0.020 a	0.65±0.038 b	0.49±0.027 c	0.51±0.026 a§	**<0.001**	**<0.001**	**0.098**	0.288
60%P_0	0.39±0.016 a	0.46±0.028 b	0.33±0.018 c	0.34±0.018 c	**<0.001**	**<0.001**	**0.046**	0.224
80%P_0	0.13±0.011 a	0.18±0.015 b	0.10±0.008 c	0.11±0.008 a	**<0.001**	**<0.001**	**0.009**	**0.087**
90%P_0	0.048±0.006 a	0.069±0.008 b	0.032±0.005 c	0.031±0.006 c	**<0.001**	**<0.001**	0.115	**0.085**
$a/P_0 \times 10^2$	2.3±0.2 a	1.8±0.1 b	2.7±0. c	2.3±0.1 a	**<0.001**	**<0.001**	**0.008**	0.987

Table S4 Velocity of Contraction Data presented as means ± standard error. **Symbols**: AC = adult control, AE = adult exercise, OC = older control, OE = older exercise, different letters indicate differences at p<0.10; " ǂ "≠ OC, p<0.05; " § "≠AE, p<0.05; " ¥ "=AE; Age = main effect of age, Training = main effect of training; Inter. = interaction of age*training, p-value, from 2x2 ANOVA, bold indicating significance; fl/s = fiber lengths/sec.

Power mN*fl/s	AC n=9	AE n=6	OC n=12	OE n=9	2x2 ANOVA p-value	ME Age	ME Training	Inter.
P_{max}[1]	89.1±4.7 a	97.7±10.5 a	54.7±5.3 b	61.9±46.5 b	**<0.001**	**<0.001**	0.240	0.918
10%P_0	61.5±4.0 a	65.1±5.5 a	42.7±3.7 b	42.7±4.0 b	**<0.001**	**<0.001**	0.396	0.983
20%P_0	81.1±5.0 a	87.2±7.3 a	53.0±5.1 b	56.8±4.8 b	**<0.001**	**<0.001**	0.382	0.845
30%P_0	85.6±5.2 a	93.5±7.7 a	54.1±5.2 b	59.9±5.0 b	**<0.001**	**<0.001**	0.245	0.860
40%P_0	81.6±4.8 a	90.7±7.5 a	51.3±5.0 b	56.8±4.7 b	**<0.001**	**<0.001**	0.196	0.743
50%P_0	72.1±4.2 a	81.9±6.9 a	44.6±4.5 b	49.7±4.1 b	**<0.001**	**<0.001**	0.144	0.635
60%P_0	59.9±3.4 a	69.1±6.1 a	35.5±3.7 b	40.0±3.3 b	**<0.001**	**<0.001**	**0.081**	0.501
80%P_0	26.1±1.7 a	36.3±3.9 b	14.5±1.8 c	17.2±1.5 c	**<0.001**	**<0.001**	**0.005**	0.217
90%P_0	10.6±1.2 a	15.5±2.1 b	5.5±1.1 c	5.4±1.0 c	**<0.001**	**<0.001**	**0.078**	**0.070**
%P_0 at P_{max}[1]	27.3±0.6 a	29.1±0.5 b	27.3±0.4 c	27.9±0.3 c	**<0.001**	0.188	**0.022**	0.188

Table S5 Power Production Data presented as means ± standard error. **Symbols**: P_{max} = maximum power output, %P_0@Pmax = the percentage of P_0 (maximum force) where P_{max} occurs; AC = adult control, AE = adult exercise, OC = older control, OE = older exercise, different letters indicate differences at p<0.10; " ǂ "≠ OC, p<0.05; " § " ≠ AE, p<0.05; " ¥ "= AE; ME Age = main effect of age, ME Training = main effect of training, Inter. = interaction of age*training, numbers are p-values from 2x2 ANOVA, bold highlighting p<0.10; mN*fl/s = milliNewtons*fiber lengths/sec; [1]n=7 for OE

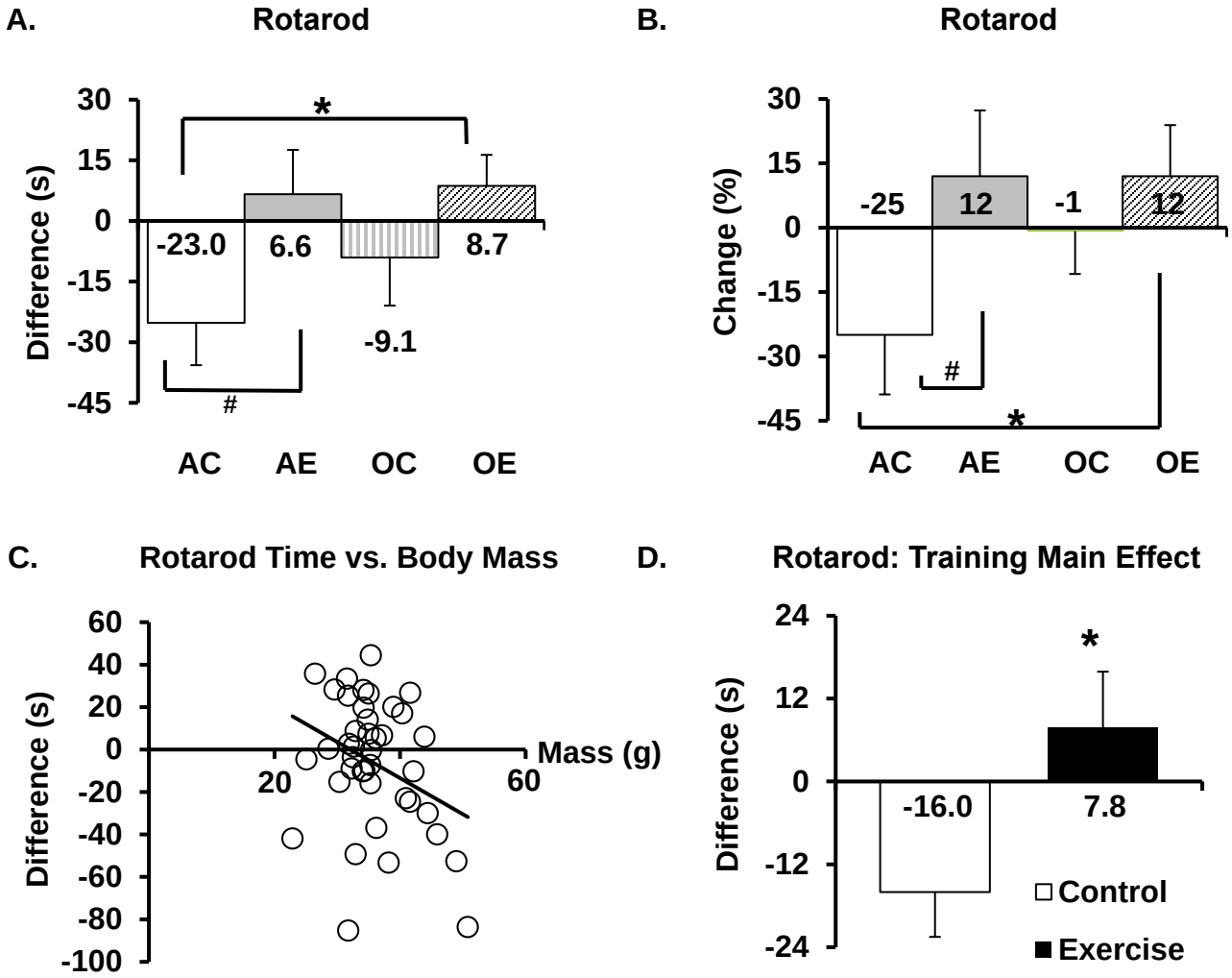

Figure S1 Rotarod Performance Improves with Training. **A**. Rotarod Difference, Adult and Older Mice **B.** Rotarod Percentage Change Improved with Training, Adult and Older Mice. % change **C.** Mass and Rotarod Difference Unrelated. Simple linear regression of difference in seconds dependent upon mass at sac (simple linear regression: y = -1.70x + 54.57 R = 0.325, p=0.053) was not statistical when all four groups were compared**. D**. Main effect of training was significant when collapsed over age. Symbols: AC = adult control, AE = adult exercise, OC = older control, OE = older exercise, s = seconds, g = grams, "#" = p<0.10, "*" = p<0.05, numbers at base of columns = means, error bars = standard error.

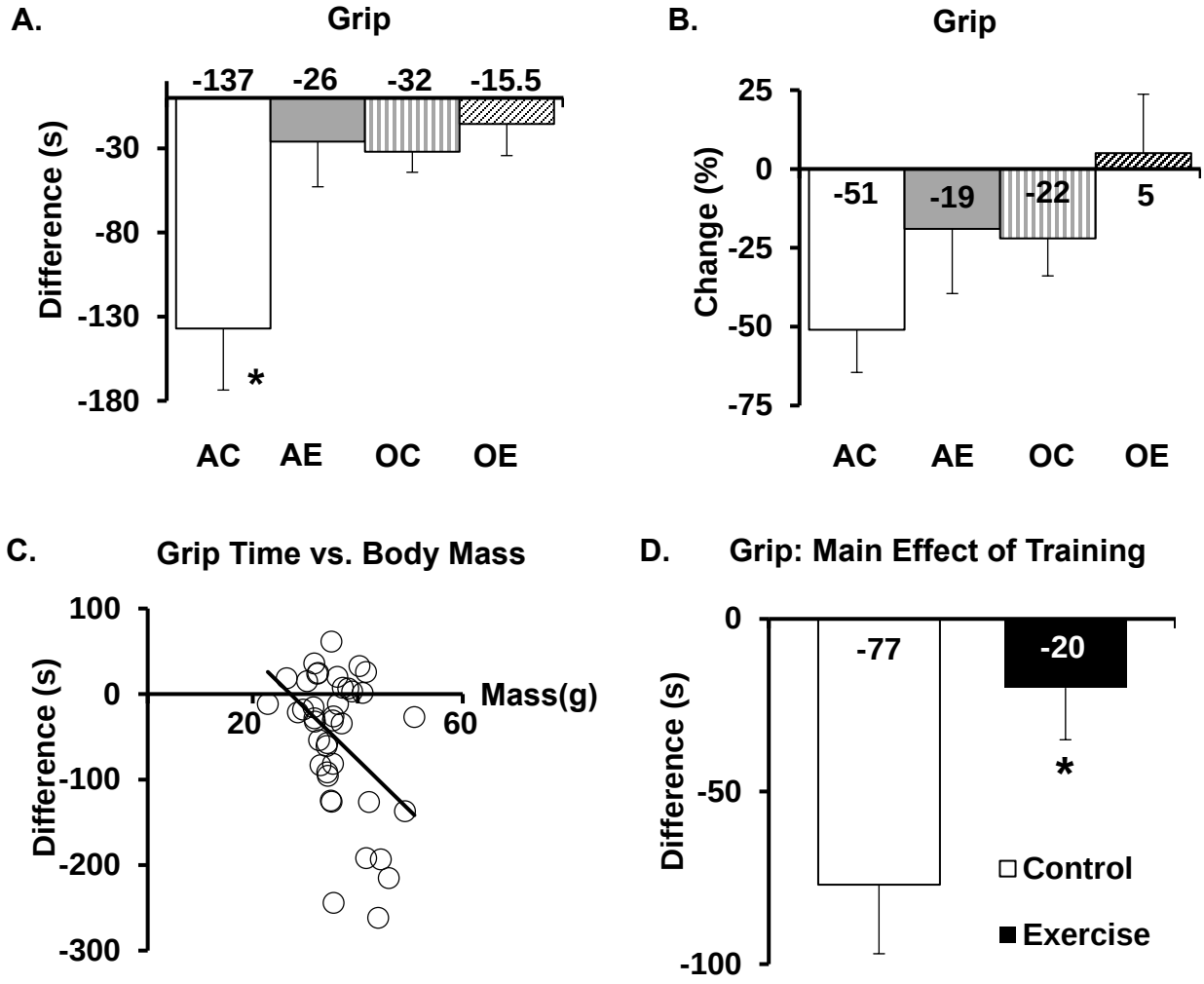

Figure S2 Grip Function Preserved with Training. A. Grip Difference (seconds) AC<AE; < OC, p=0.043; <OE, p=0.017; adjusted for body mass. **B.** Grip Percent Change: no significant difference. **C.** Grip difference in seconds was correlated with mass at sacrifice (simple linear regression: y = -6.00x + 163.26, R = 0.425, p=0.010). D. Main Effect Exercise (grip seconds) p=0.027. AC = adult control, AE = adult exercise, OC = older control, OE = older exercise. Numbers at bar = mean; error bars = standard error, s = seconds, g =grams, * = p<0.05.

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